Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1-30 are pending in the application, with claim 1 being the independent claim. Support for amendments to claim 1 may be found at page 31, lines 11 and 12, page 34, lines 4-6, and at page 12, lines 12-16 of the specification. Support for amendments to claim 2 may be found at page 34, lines 4-6 of the specification. Support for amendments to claim 4 may be found at page 31, lines 11 and 12 of the specification. Support for amendments to claims 7 and 8 may be found at page 8, lines 8-10, and page 12, lines 17-23, respectively. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Objection to the Specification

The Examiner has objected that the nucleotide sequences listed on page 27 of the specification lack sequence identifiers as required under 37 C.F.R. 1.821(d). The Applicant has amended the specification to include the identifiers and further submits herewith an amended paper and computer readable copy of the sequence listing that incorporates the nucleotide sequences.

Rejections under 35 U.S.C. § 112

The Examiner has objected to claims 1-30 under 35 U.S.C. § 112, first paragraph, stating it is not clear that the nucleic acid molecule of claim 1 is freely available or can be reproducibly isolated from nature, and thus the application fails to meet the requirements for enablement. Accordingly, the Examiner requests certain assurances regarding a biological deposit of the bacterial strain containing the endogenous plasmid. Applicants respectfully traverse this rejection.

Applicants submit herewith a Statement Concerning the Deposited Plasmid DNA, filed on March 29, 2002, during prosecution of U.S. Patent No. 6,503,748, which provides assurances that strain NRRL B-30035 has been deposited under terms of the Budapest Treaty and that all restrictions on the availability to the public of the deposit will be irrevocably removed upon the granting of a patent. Accordingly, withdrawal of the rejection is respectfully requested.

The Examiner rejected claims 1 and claims 2-30, under 35 U.S.C. § 112, second paragraph, as allegedly indefinite for not providing the specific region at which the replicon sequence begins and ends, and that without such identifying characteristics, it would be impossible for the Examiner to search for sequences that are 95% identical to the replicon sequence. Applicant has amended claim 1. Applicant respectfully traverses this rejection as it may be applied to the pending claims.

Amended claim 1 is drawn to an isolated or purified nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a nucleotide sequence of a *Ketogulonigenium* plasmid replicon found on the endogenous plasmid contained in Deposit No. NRRL B-30035, wherein the replicon comprises SEQ

ID NO:4 or a fragment thereof, wherein said fragment is capable of functioning as a replicon in *Ketogulonigenium*. Applicant asserts that the claim satisfies the Examiner's requirement that it provide specific nucleotide sequences for the purpose of conducting a search. The Applicant further asserts that nucleotide fragments of SEQ ID NO:4 should also be encompassed by the claims, provided the fragments are capable of autonomous replication in *Ketogulonigenium*. The Applicant proffers the following reasons in support of the assertions.

The Applicant has identified SEQ ID NO: 4, a sequence found on an endogenous plasmid contained in NRRL B-30035 that autonomously replicates in *Ketogulonigenium*. Applicant has further identified a fragment of SEQ ID NO:4, namely SEQ ID NO:1, that is also able to direct replication in *Ketogulonigenium*. Thus, Applicant has demonstrated that he possesses a fragment of SEQ ID NO:4 that is capable of autonomous replication and further, demonstrates the principle that an autonomously replicating sequence may be reduced to a smaller fragment, also capable of autonomous replication. Laboratory procedures to obtain smaller fragments of SEQ ID NO:1 may also be performed, to identify the minimal nucleotide sequence that is sufficient to direct replication in *Ketogulonigenium*. However, such procedures would provide little, if any, substantive benefit to the Applicant for the purpose directing replication in *Ketogulonigenium*. On the other hand, if the Applicant were not allowed to claim such functional fragments, a third party could identify smaller fragments that potentially fall outside the scope of the Applicant's claims and practice the substance of the invention without literally infringing the claims. Therefore, Applicant deems it necessary that the claims encompass

functional fragments of SEQ ID NO: 4. Accordingly, withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. § 112, Second Paragraph

The Examiner further rejected claim 7, and claims 8-9 under 35 U.S.C. § 112, second paragraph, as allegedly indefinite for recitation of the phrase "mob region". The Examiner alleged that the specification does not provide a definition of "mob region," thus rendering the claim indefinite. Applicant has amended claims 7 and 8. Applicant respectfully traverses this rejection as it may be applied to the pending claims.

Applicant has amended claim 7 and 8 by replacing "mob region" with "mob site." Applicant respectfully directs the Examiner's attention to the specification:

> The mob site includes the origin of transfer (*ori*T) and acts as a recognition site for certain *trans* active plasmid transfer functions (R. Simon *et al.*, *Bio/Technology 1*:784-791 (1983). A mob region is usually a *mob* gene and an *ori*T from any conjugation plasmid. The mob site can be obtained from a conjugative plasmid, e.g., plasmids RK2, RP4, RSF1010, or plasmids belonging to incompatibility groups IncP, IncQ, IncC, IncB, IncF, IncG, IncI, IncK, IncM, IncN, IncPa, IncPb, IncW, IncX, and IncZ or their derivatives.

Specification, at page 12, lines 17-23.

A "mob site" as referred to in the specification is a region of DNA necessary for conjugal transfer and that acts as a recognition site for certain *trans* active plasmid transfer functions. Thus, "mob site" is defined by the specification. Accordingly, Applicant respectfully requests that the Examiner reconsider and withdraw that rejections.

The Examiner further rejected claim 21 under 35 U.S.C. § 112, first paragraph, as allegedly indefinite because there is not sufficient antecedent basis for "E.coli derived

plasmid." Applicant has amended claim 21. Applicant respectfully traverses this rejection as it may be applied to the pending claim.

Applicant has amended the claim so that it now depends from claim 20, where there is antecedent basis for support. Accordingly, Applicant respectfully requests that the Examiner reconsider and withdraw that rejections.

The Examiner further rejected claim 23 under 35 U.S.C. § 112, first paragraph, as allegedly indefinite because there is not sufficient antecedent basis for "reporter gene." Applicant has amended claim 23. Applicant respectfully traverses this rejection as it may be applied to the pending claim.

Applicant has amended the claim so that it now depends from claim 22, where there is antecedent basis for support. Accordingly, Applicant respectfully requests that the Examiner reconsider and withdraw that rejections.

The Examiner rejected claim 24 under 35 U.S.C. § 112, first paragraph, as allegedly indefinite for reciting the phrase "and at least one organism." Applicant has amended claim 24. Applicant respectfully traverses this rejection as it may be applied to the pending claim.

The Applicant has amended the claim based on the Examiner's suggestion to recite "and at least in one organism." Accordingly, Applicant respectfully requests that the Examiner reconsider and withdraw that rejections.

Other Matters

Applicant thanks the Examiner for initialing and returning the PTO-1449 forms. Applicant respectfully requests that the Examiner consider the document AM3, EP 0278447, which was not initialed.

Applicant requests that the Examiner delete the phrase "encoding the pyruvate carboxylase polypeptide" from paragraph [0017] in the specification. This phrase is an obvious error in the specification and the artisan would immediately recognize that a reference nucleotide that is referred to in this paragraph is not construed to represent a nucleotide encoding pyruvate carboxylase polypeptide. For example, in the sentence following the phrase, the specification reads:

> In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence.

Specification at page 6, lines 8-12.

The sentence refers to "a reference nucleotide sequence" and does not refer back to the antecedent reference pyruvate carboxylase polypeptide. For these reasons, one of ordinary skill in the art would recognize the error. Secondly, the application concerns nucleotide sequences derived from endogenous plasmids that function as replicons. One of skill in the art would distinguish replicon sequences from sequences encoding a pyruvate carboxylase polypeptide. Therefore, owing to the obvious error, the Applicant respectfully requests that the Examiner delete this phrase from the paragraph.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Michele A. Cimbala

Michele A. Cimbala
Attorney for Applicant
Registration No. 33,851

Date: Jan. 22, 2003

1100 New York Avenue, N.W.
Suite 600
Washington, D.C. 20005-3934
(202) 371-2600

Version with markings to show changes made

In the Specification:

Please substitute pending paragraph [0017] with the following paragraph [0017]:

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence [encoding the pyruvate carboxylase polypeptide]. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence.

Please substitute pending paragraph [0073] with the following paragraph [0073]:

Forward and reverse oligonucleotide primers were designed with which to initiate PCR reactions using purified pADM291 DNA as substrate. The oligonucleotide primers were prepared by a commercial lab (Sigma/Genosys, The Woodlands, TX) using established methods known in the art. The primers had the following features ("F"=forward primer; "R"=reverse primer):

Table I

Primer Label	DNA Sequence
Primer 1F	CGGAATTCGATCATATCATTCCCCAAGCGGAC (SEQ ID NO:5)
Primer 1R	GCTCTAGACGATGTACTCCTTGGTGCTCTCGAT (SEQ ID NO:6)
Primer 2F	CGGAATTCTGCTTCTTTTCGTTCGTTTCCGCC (SEQ ID NO:7)
Primer 2R	GCTCTAGAACATCGCTCATCTGTAGTCGCC (SEQ ID NO:8)
Primer 3F	CGG AATTCACAGTCAGGTGGCACATGTTCC (SEQ ID NO:9)
Primer 3R	CGG GATCCTGTGAAAAAGTGAGGACAGGCGGG (SEQ ID NO:10)
Primer 4F	CGGAATTCGGCAATGGGTCGAAATTCATAG (SEQ ID NO:11)
Primer 4R	CGGGATCCACGTTCCCTATTTTCCTCAATC (SEQ ID NO:12)
Primer 5F	CGGAATTCACACCGAAACACCTAACACGCAAG (SEQ ID NO:13)
Primer 5R	CGGGATCCAGTGCGGTTCACGTCATCAATG (SEQ ID NO:14)
Primer 6F	CGGAATTCTGCACTGCCGCTCTCGAAATG (SEQ ID NO:15)
Primer 6R	CGGAATTCACAAGATTGACGCAGCTCTTCGC (SEQ ID NO:16)
Primer 7F	CGGAATTCGGCAATGGGTCGAAATTCATAG (SEQ ID NO:17)
Primer 7R	CGCGGATCCACTTGTGTTGTCTTTCCC (SEQ ID NO:18)
Primer 8F	GCGGAATTCGACGCTGCAAACATCGAAAAAC (SEQ ID NO:19)
Primer 8R	CGGGATCCACGTTCCCTATTTTCCTCAATC (SEQ ID NO:20)

In the Claims:

Please substitute the following claim 1 for the pending claim 1:

1. (once amended) An isolated or purified nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a nucleotide sequence of a *Ketogulonigenium* plasmid replicon found on the endogenous plasmid contained in Deposit No. NRRL B-30035[.], wherein said replicon comprises SEQ ID NO:4 or a fragment thereof, wherein said fragment is capable of functioning as a replicon in *Ketogulonigenium*.

Please substitute the following claim 2 for the pending claim 2:

2. (once amended) The nucleic acid molecule of claim 1, wherein said [plasmid] replicon comprises said fragment, wherein said fragment comprises the nucleic acid sequence in SEQ ID NO:1.

Please substitute the following claim 4 for the pending claim 4:

4. (once amended) The nucleic acid molecule of claim 1, wherein said replicon comprises [comprising] the DNA sequence shown in SEQ ID NO:4.

Please substitute the following claim 7 for the pending claim 7:

7. (once amended) The nucleic acid molecule of claim 1, comprising a mob [region] site.

Please substitute the following claim 8 for the pending claim 8:

8. (once amended) The nucleic acid molecule of claim 7, wherein said mob [region] site comprises a mob gene and an oriT from a conjugation plasmid.

Please substitute the following claim 21 for the pending claim 21:

21. (once amended) The nucleic acid molecule of claim [19] 20, wherein said E. coli-derived plasmid is selected from the group [comprising] consisting of pET, pUC18, and pUC19.

Please substitute the following claim 23 for the pending claim 23:

23. (once amended) The nucleic acid molecule of claim [21] 22, wherein said reporter gene encodes a protein selected from the group consisting of ß-galactosidase, ß-glucuronidase, luciferase, green fluorescent protein α-amylase, and uroporphyrinogen III methyltransferase (cobA) from *Propionibacterium freudenreichii*.

Please substitute the following claim 24 for the pending claim 24:

24. (once amended) The nucleic acid molecule of claim 1, wherein said nucleic acid molecule autonomously replicates in *Ketogulonigenium* and in at least one organism selected from the genera consisting of *Acetobacter, Corynebacterium, Bacillus, Rhodobacter, Paracoccus, Roseobacter, Pseudomonas, Pseudogluconobacter, Gluconobacter, Serratia, Mycobacterium,* and *Streptomyces*.